Chapter VI
Copolymers of Covalently Crosslinked Linear and Branched PEIs as Efficient Nucleic Acid Carriers
In vitro and In vivo
1. Introduction

In the previous chapters, both linear and branched PEIs were modified using different methodologies and evaluated in terms of gene delivery ability. The high efficiency of bPEI as a gene delivery vector is due to its excellent buffering capacity in the pH range 3-5, which helps in its escape from the endosomes (Sparks et al., 2011; Lee et al., 2011). PEI exists in two forms, viz., branched and linear with varying molecular weights ranging from 0.2kDa to 750kDa. High molecular weight and branching i.e., a particular ratio of 1º, 2º, and 3º amines is necessary for effective gene transfection with PEI. But, high molecular weight and presence of excess primary amines bring with themselves a big hurdle in transfection, i.e., toxicity. Conversely, linear PEIs show poor gene transfection efficiency with much lower cytotoxicity. Therefore, to maintain a balance between efficiency and cytotoxicity, a reasonable way is to combine the properties of the two PEIs i.e., linear and branched forms. Thomas et al. (2005) crosslinked low molecular weight PEIs via ester and amide bearing linkages that resulted in the improved transfection efficiency, i.e., ~2 folds over bPEI (25kDa). But this strategy led to the masking of positive charge of PEI by decreasing the total number of amines by amide formation. Subsequently, Peng et al. (2009) studied the effect of disulfide density and molecular weight on disulfide crosslinked bPEIs as gene delivery vectors. The crosslinked bPEIs with lowest molecular weight (800Da) gave the best results among all the PEIs tested. Although the results were promising in terms of transfection efficiency, the IC₅₀ values decreased drastically on increasing the thiolation degree even in case of PEI800 (Peng et al., 2009), which is indicative of high toxicity.

The present study was undertaken to address the limitations of the previous studies by conjugating linear and branched PEIs (25kDa each) using an epoxy linker. By varying the ratio of lPEI to bPEI, a series of crosslinked copolymers (bPEI-lPEI, BL) was prepared and evaluated for their transfection efficiency and cytotoxicity. Having characterized by physico-chemical techniques, the gene carrying ability of these copolymers was assessed on HEK293, HeLa and CHO cell lines and simultaneously determined the toxicity of these copolymers. Among the series, BL 2:1(15%) displayed significantly higher transfection efficiency as compared to native PEIs and commercial reagents tested in the study. In vivo gene expression analysis using BL 2:1(15%) exhibited maximum expression in the spleen tissue of mice.
2. Experimental section

2.1. Preparation of crosslinked PEI copolymers

A solution of lPEI (50mg, 1mg/ml) in dd water was heated to 90°C and after complete dissolution, epichlorohydrin (2.3µl, for 5% substitution) was added. The reaction mixture was stirred for 4h at ~60°C. For 1:1 crosslinking of lPEI and bPEI, a solution of bPEI (50mg, 1mg/ml) was added at the same temperature. Then, a solution of sodium hydroxide (1N, 2ml) was added drop wise and allowed to stir the reaction mixture for 4h at the same temperature. Volume was reduced (up to one third) through rotary evaporation and dialyzed the reaction mixture against water (24h, till the solution became neutral) and lyophilized to obtain BL 1:1 (5%) in ~85% yield. Likewise, BL 1:1 with 10, 15 and 20% crosslinking were prepared in ~80-85% yields. Similarly, BL 2:1, 3:1, 4:1 and 5:1 series (with increasing ratio of bPEI) were prepared with varying percentage (5, 10, 15, and 20) of crosslinking with ~80-85% yields.

2.2. Formation of copolymer/DNA complexes

To form copolymer/DNA complexes, 1µl of DNA (0.3µg/µl) was added to an aqueous solution of BL copolymer (1.0mg/ml) at various w/w ratios of copolymer/DNA (0.66, 1.0, 1.66, 2.33) and the final volume was made up to 20µl with water. For in vitro transfection assays, 5µl of 20% dextrose solution was added before making up the final volume to 20µl with water. The resulting samples were gently vortexed and incubated for 30min at 25±1°C prior to their use in biophysical studies or transfection experiments.

2.3. Physical characterization of copolymers

The prepared copolymers were characterized for their morphology, size and zeta potential. The hydrodynamic diameter of BL copolymers (1mg/ml) and their DNA complexes, suspended in water and 10% serum, were measured by DLS, as described in Chapter II.

2.4. DNA retardation assay

Branched and linear PEIs and BL copolymers were complexed with pDNA (0.3µg/µl) at w/w ratio 0.15, 0.33, 0.66 and 1.0. DNA complexes (20µl) were mixed with 2µl xylene cyanol (in 20% glycerol) and electrophoresed (100V, 1h) on 0.8% agarose gel, as described in Chapter II.

2.5. Toxicity studies

Cytotoxicity of PEIs/pDNA, BL/pDNA, as well as Lipofectamine\textsuperscript{TM}/pDNA complexes was evaluated on HEK293, CHO and HeLa cells by MTT colorimetric assay following the procedure described in Chapter II. IC\textsubscript{50} value, at which the HEK293 cell viability reaches 50%, was estimated for BL 2:1/DNA and PEI/DNA complexes at w/w ratio of 1.0. The transfection efficiency of BL copolymers and PEIs was found to be highest at these w/w ratios.
2.6. **DNase I protection assay**

For assessing the ability of BL 2:1(15%) copolymers to protect the condensed pDNA from nucleases, DNase I protection assay was performed, as described in Chapter II.

2.7. **DNA release assay**

PEIs and BL copolymers were complexed with pDNA (300ng) at w/w ratio where these complexes exhibited the highest transfection efficiency and incubated for 30min, as described above. Heparin was added in different amounts varying from 0.1-12U for the release of plasmid DNA and the remaining steps were performed following the procedure described in Chapter II.

2.8. **Buffering Capacity**

A suspension of BL 2:1(5%) (6mg/30ml) in 0.1N NaCl was adjusted to pH 10 with 0.1N NaOH and then the pH was brought to 3.0 with addition of 50μl aliquots of 0.1N HCl. pH values were recorded after each addition. The slope of the line in the plot for pH and the amount of HCl consumed indicates the intrinsic buffering capacity of the system. Similarly, the projected assay was repeated with BL 2:1(10%), BL 2:1(15%), BL 2:1(20%) and PEIs.

2.9. **In vitro transfection assay**

The transfection experiments involving BL, IPEI, bPEI and Lipofectamine™/DNA complexes, in the presence and absence of serum, were performed on HEK293, CHO and HeLa cells, as described in Chapter II.

Delivery of siRNA on HEK293 cells was evaluated using BL 2:1(15%) copolymer following sequential delivery approach and compared the results with Fugene™, as described in Chapter II.

2.10. **Quantification of EGFP expression**

EGFP expression in mammalian cells was quantitatively estimated on NanoDrop ND-3000 spectrofluorometer following the procedure described in Chapter II.

2.11. **Fluorescence-activated cell sorting (FACS) analysis**

For examining GFP expression at the individual cell level, FACS analysis was performed at 36h post-transfection. Briefly, HEK293 cells, after seeding in 24-well plates for 16h, were washed with phosphate buffer saline (PBS) following aspiration of the medium. PEIs and BL 2:1 series were complexed with pDNA (1.5µg) at w/w ratios of 0.66, 1.0, 1.66, 2.33, 3.33 and incubated for 30min at 25±1°C. Similarly, pDNA complex was prepared with Lipofectamine™ following manufacturer’s protocol. The assay was performed according to the procedure described in Chapter II.
2.12. Confocal laser scanning microscopy (CLSM)

Intracellular trafficking of labeled BL 2:1(15%)/DNA complex in HeLa cells was monitored using confocal microscopy according to the procedure described in Chapter II.

2.13. Intravenous injection of BL 2:1 (15%)/DNA complex in Balb/c mice

Luciferase vector, pGL3 (25µg) was complexed with BL 2:1(15%) and bPEI at a w/w ratio of 1 with a final volume up to 100µl using normal saline. The complexes were incubated for 30min at 25±1°C. Similarly, naked DNA (25µg) in 100µl normal saline was incubated for 30min at 25±1°C. Three Balb/c mice were injected with the above made complexes i.e., BL 2:1(15%)/DNA, bPEI/DNA and naked DNA through tail vein using a syringe of 40U (insulin syringe, 1ml) with needle of size 0.30 x 8mm. After 7 days on normal diet, the animals were sacrificed and the luciferase activity in different organs was quantified according to the procedure described in Chapter II.

3. Results & discussion

Branched PEI (25kDa) displays high transfection efficiency, however, associated with significant toxicity. Likewise, linear PEI (25kDa) is almost non-toxic, however, exhibits low transfection efficiency due to poor solubility in water. In order to design a polymer possessing the transfection efficiency of bPEI and toxicity comparable to lPEI, it was decided to crosslink lPEI with bPEI using a crosslinker that would not block the amines on either of the polymers. Therefore, epichlorohydrin was selected, which can react with both 1º and 2º amines of linear and branched PEIs. The chemistry of epichlorohydrin is well known and has been used by the researchers for various modifications and subsequent applications (Jug et al., 2011; Yi et al., 2011, Yun et al., 2011). During crosslinking, this reaction offered several advantages, viz., i) it converted some of the toxic primary amines of bPEI into secondary or tertiary to make it less toxic, ii) simultaneously, it resulted in the increased buffering capacity, iii) the copolymers, so obtained, were found to be of nanosize, which improved the cellular uptake, and iv) it helped in overcoming the solubility issue related to lPEI. The unique advantage of using epichlorohydrin as a crosslinking agent inking group is that it does not block the overall charge but merely converts one form (bad) into another (good), thus maintaining the total number of amines intact.

3.1. Preparation, purification and characterization of BL copolymers

The preparation of BL series is shown in Scheme 1. To prepare BL series, first, lPEI was converted into its chlorohydrin derivative (CHL) with the reaction of varying amounts of
epichlorohydrin and subsequently reacted with bPEI in basic medium by *in situ* generation of epoxy functionalities. The chlorohydrin derivative of IPEI was taken as a template for the subsequent crosslinking with bPEI, the reason for which lies in the fact that IPEI contains only secondary amines all over the chain and has solubility issue at room temperature. Once chlorohydrin derivative has been formed, there are chances for both inter- and intramolecular reactions upon addition of the second polymer. However, as bPEI contains 1° amines, which are more reactive than secondary ones, there are fair chances that only intermolecular reaction will take place. Moreover, to confirm this logic, this reaction was also carried out by taking bPEI as the template, however, the resulting copolymers were found to be insoluble using this strategy. Therefore, copolymers were prepared following the first route i.e., addition of bPEI onto IPEI-CH.

![Scheme 1](image.png)

**Scheme-1**: Schematic presentation of preparation of BL copolymers

### 3.2. Mobility shift assay

Positive charge on cationic polymer and negative phosphate backbone of DNA interact with each other through electrostatic interactions and form complexes. DNA
retardation assay was performed to find out the amount of cationic co-polymer required to retard the mobility of fixed amount of DNA. Figure 1 shows DNA binding to BL copolymers at different w/w ratios. It was observed that a complete retardation of 0.3µg of pDNA-native PEI complex occurred at w/w ratio of 0.66:1, while BL copolymers retarded at w/w ratio ranging from 0.66:1-1:1. This might be due to the fact that after crosslinking total number of amines are not getting altered but only one form of charge is getting converted into another form.
Figure-1: DNA retardation assay of BL copolymers/DNA, and PEIs/DNA complexes.

3.3 Buffering capacity

Buffering capacity of the BL 2:1 series was determined using an acid-base titration in the pH range 3-10. Figure 2 shows the buffering capacity of different BL copolymers. It was found that proton capturing tendency of the copolymers was slightly decreased when compared to PEIs. This might be due to the fact that during crosslinking of two PEIs, some of the amines are embedded inside the nanosized structure and are not freely available for the protonation. Also, on increasing the percent crosslinking within the series, buffering capacity did not alter and all the copolymers almost behaved in a similar fashion.

3.4 Size and zeta potential measurements

The particle sizes of the BL copolymers and BL/DNA complexes were determined by DLS in water and 10% serum and shown in table 1. The particle size of BL copolymers ranged between 112-968nm. On complexation with DNA, particle size decreased due to formation of more compact structures and ranged between 100-400nm. Moreover, the particle size further decreased in presence of 10% serum and ranged between 25-176nm. The percent crosslinking was also found to affect the size of copolymers and showed a decreasing trend with increasing crosslinking in a particular series. Also, as the weight ratio of bPEI in the copolymers increased, the size also increased due to attachment of higher number of bPEI units.
Table-1: Particle size and zeta potential measurements of BL copolymers and their corresponding DNA complexes in water and serum at w/w ratio of 1:1.

<table>
<thead>
<tr>
<th>Size</th>
<th>Copolymer (nm)</th>
<th>With DNA (nm)</th>
<th>With Serum (nm)</th>
<th>Copolymer (mV)</th>
<th>With DNA (mV)</th>
<th>With serum (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL 1:1 (5%)</td>
<td>301.1</td>
<td>186.7</td>
<td>29.9</td>
<td>24.5</td>
<td>29.7</td>
<td>-11.6</td>
</tr>
<tr>
<td>BL 1:1 (10%)</td>
<td>215.8</td>
<td>155.9</td>
<td>36.7</td>
<td>18.1</td>
<td>12.1</td>
<td>-12.2</td>
</tr>
<tr>
<td>BL 1:1 (15%)</td>
<td>118.2</td>
<td>154.0</td>
<td>34.1</td>
<td>16.7</td>
<td>10.5</td>
<td>-14.1</td>
</tr>
<tr>
<td>BL 1:1 (20%)</td>
<td>112.3</td>
<td>101.6</td>
<td>30.6</td>
<td>14.2</td>
<td>5.0</td>
<td>-14.0</td>
</tr>
<tr>
<td>BL 2:1 (5%)</td>
<td>558.8</td>
<td>265.3</td>
<td>26.0</td>
<td>25.0</td>
<td>13.7</td>
<td>-12.6</td>
</tr>
<tr>
<td>BL 2:1 (10%)</td>
<td>470.6</td>
<td>185.0</td>
<td>29.5</td>
<td>19.5</td>
<td>12.0</td>
<td>-13.5</td>
</tr>
<tr>
<td>BL 2:1 (15%)</td>
<td>378.7</td>
<td>180.1</td>
<td>37.0</td>
<td>22.8</td>
<td>11.8</td>
<td>-12.1</td>
</tr>
<tr>
<td>BL 2:1 (20%)</td>
<td>298.3</td>
<td>127.8</td>
<td>34.6</td>
<td>18.8</td>
<td>4.3</td>
<td>-12.5</td>
</tr>
<tr>
<td>BL 3:1 (5%)</td>
<td>642.1</td>
<td>205.8</td>
<td>27.1</td>
<td>28.3</td>
<td>17.6</td>
<td>-12.5</td>
</tr>
<tr>
<td>BL 3:1 (10%)</td>
<td>487.2</td>
<td>161.6</td>
<td>25.9</td>
<td>29.9</td>
<td>9.8</td>
<td>-14.0</td>
</tr>
<tr>
<td>BL 3:1 (15%)</td>
<td>365.8</td>
<td>152.5</td>
<td>28.9</td>
<td>24.1</td>
<td>9.3</td>
<td>-14.4</td>
</tr>
<tr>
<td>BL 3:1 (20%)</td>
<td>134.1</td>
<td>127.5</td>
<td>34.4</td>
<td>19.0</td>
<td>6.7</td>
<td>-12.8</td>
</tr>
<tr>
<td>BL 4:1 (5%)</td>
<td>590.8</td>
<td>276.2</td>
<td>44.3</td>
<td>30.1</td>
<td>8.1</td>
<td>-14.5</td>
</tr>
<tr>
<td>BL 4:1 (10%)</td>
<td>445.9</td>
<td>145.3</td>
<td>47.4</td>
<td>28.6</td>
<td>7.9</td>
<td>-13.1</td>
</tr>
<tr>
<td>BL 4:1 (15%)</td>
<td>365.2</td>
<td>129.6</td>
<td>46.3</td>
<td>25.5</td>
<td>6.8</td>
<td>-14.1</td>
</tr>
<tr>
<td>BL 4:1 (20%)</td>
<td>219.4</td>
<td>112.8</td>
<td>47.2</td>
<td>22.0</td>
<td>5.2</td>
<td>-12.4</td>
</tr>
<tr>
<td>BL 5:1 (5%)</td>
<td>908.4</td>
<td>398.2</td>
<td>176.2</td>
<td>31.3</td>
<td>5.6</td>
<td>-9.8</td>
</tr>
<tr>
<td>BL 5:1 (10%)</td>
<td>778.1</td>
<td>267.9</td>
<td>160.9</td>
<td>28.8</td>
<td>2.8</td>
<td>-10.6</td>
</tr>
<tr>
<td>BL 5:1 (15%)</td>
<td>742.8</td>
<td>245.1</td>
<td>124.8</td>
<td>27.5</td>
<td>1.9</td>
<td>-13.3</td>
</tr>
<tr>
<td>BL 5:1 (20%)</td>
<td>437.7</td>
<td>197.2</td>
<td>72.9</td>
<td>25.1</td>
<td>1.2</td>
<td>-18.5</td>
</tr>
<tr>
<td>bPEI</td>
<td>-</td>
<td>208.8</td>
<td>98.7</td>
<td>35.1</td>
<td>15.8</td>
<td>-10.8</td>
</tr>
</tbody>
</table>
Zeta potential showed a decreasing trend on increasing the percentage crosslinking within the series. While on increasing the weight ratio of bPEI:lPEI, the zeta potential was found to increase. This might be due to the fact that, within the series as the percentage of crosslinking is increased, some of the primary and secondary amines are getting converted into secondary and tertiary ones, respectively. This also leads to some charge masking, which is not available at the surface. But as we increase the amount of bPEI in the series, more number of primary amines are available on the surface, which may be responsible for enhanced positive charge of the copolymers. Further, on complexation with pDNA, the zeta potential decreased, which might be due to neutralization of positive charge by the negative phosphate backbone.

3.5. Protection of pDNA against nucleases

To achieve successful entry of DNA in a cell through transfection, its protection from nucleases is a pre-requisite since these nucleases rapidly degrade native DNA. Therefore, DNase I assay was carried out to demonstrate the efficacy of BL 2:1(15%) towards protecting pDNA. Naked DNA was found to be digested by DNase I within 15min, whereas in BL 2:1(15%)-pDNA complex, ~87% pDNA was isolated intact even after 2h of treatment (Fig. 3). These observations clearly suggest that BL 2:1(15%) can be used as an efficient vector for taking the DNA to the cellular milieu without much of degradation.

![Figure 3: DNase I protection assay.](image)

3.6. DNA binding ability

bPEI is known to bind DNA very tightly, which may eventually affect the release of DNA during transfection. While on the other hand, lPEI binds DNA very loosely. In order to compare the pDNA binding ability of BL 2:1(15%) with bPEI and lPEI, heparin release assay was performed. After using an increasing concentration of heparin (0.1-12U) in the reaction mixture, a maximum release (57%) of DNA was observed in presence of 12U of heparin by
bPEI. Under similar conditions, lPEI released almost all the complexed DNA (95%) (Fig. 4). Interestingly, the binding ability of BL 2:1(15%) was observed to lie between bPEI and lPEI, i.e., it was able to release the pDNA upto 80% with the use of 12U of heparin. The results implied that BL 2:1(15%)/DNA complex was neither too tightly nor loosely bound. Moreover, it might be due to the excessive positive charge on bPEI as well as its open structure, which is responsible for tight binding of DNA, whereas, copolymers are compact.

![Figure-4: DNA release assay of PEIs and BL copolymers.](image)

3.7. Toxicity assessment

MTT assay was carried out to determine the cytotoxicity of the BL series of copolymers and comparison was made with native PEIs and Lipofectamine™. It was observed that, 1:1 series exhibited least cytotoxicity (2-10%) and the toxicity slightly increased (P>0.05) as on increasing the weight ratio of bPEI in the series (~30% for 5:1 series). Interestingly, the toxicity decreased on increasing the percent crosslinking of chlorohydrin within the series. Also, the BL series exhibited lower cytotoxicity (P<0.05) compared to bPEI (~40-50%) and Lipofectamine™ (~50-55%) regardless of the percent crosslinking and ratio of two PEIs, in all the cell lines tested in the study (Fig. 5). Moreover, the cell viabilities were higher (P>0.05), similar to that of IPEI (~10-14%) in BL series. IC₅₀ values were calculated for BL 2:1 series and compared the results with bPEI and lPEI. The values indicated very high toxicity of bPEI even at very low concentrations (18mg/L) compared to BL 2:1(15%) (338mg/L) and IPEI (379mg/L) (Table-2). This shows that BL copolymers do not possess toxicity at even higher concentrations and are safer at higher doses also.
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**Figure-5**: Cell viability profile of BL copolymers/DNA, PEIs/DNA and Lipofectamine™/DNA complexes in HEK293, HeLa and CHO cells.

**Table-2**: IC₅₀ values of BL 2:1 series compared to PEIs

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC₅₀ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL 2:1(5%)</td>
<td>298</td>
</tr>
<tr>
<td>BL 2:1(10%)</td>
<td>312</td>
</tr>
<tr>
<td>BL 2:1(15%)</td>
<td>338</td>
</tr>
<tr>
<td>BL 2:1(20%)</td>
<td>350</td>
</tr>
<tr>
<td>bPEI</td>
<td>18</td>
</tr>
<tr>
<td>IPEI</td>
<td>379</td>
</tr>
</tbody>
</table>

3.8. *In vitro transfection in mammalian cells*

The gene delivery efficiency of the BL series was evaluated by *in vitro* transfection experiments onto HeLa, HEK293, and CHO cells. The results were compared with bPEI, IPEI and commercial transfection reagents. The transfection results of all the cell lines tested are presented in figure 6. It was observed that in the BL series, BL 2:1(15%) showed significantly enhanced transfection efficiency (~3.3 to 20 folds) than bPEI, IPEI and Lipofectamine™. The transfection efficiency was found to be cell line dependent and showed maximum transfection on HEK293 cells. Also, the transfection efficiency was found to depend on w/w ratio of polymer/DNA. bPEI/DNA complexes exhibited the highest transfection efficiency at w/w ratio of 1.66, IPEI/DNA at 3.33 and BL 2:1(15%)/DNA at 1. These results show that BL series requires a relatively lower charge ratio than native PEIs to be effective for efficient transfection.
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**Figure 6**: GFP fluorescence intensity in HEK293, HeLa and CHO cells in (a) absence of serum, and (b) presence of serum, transfected with BL copolymers/DNA, PEIs/DNA and Lipofectamine™/DNA complexes. The transfection profiles show fluorescence intensity expressed in terms of arbitrary units/mg of total cellular protein. The results represent the mean of three independent experiments performed in triplicates. *P<0.05 vs PEIs and Lipofectamine™.

FACS analysis was carried out to determine the exact percentage of cells expressing GFP. It was found that BL 2:1(15%)/DNA complex transfected ~67% cells at w/w ratio of 1, while bPEI, lPEI and Lipofectamine™ transfected only ~29%, ~20% and ~13% cells, respectively (Fig. 7). The results advocate the potential of these copolymers as a promising gene carrier.
Figure-7: Percent transfection efficiency of BL 2:1 copolymers/DNA complexes determined using FACS in HEK293 at various w/w ratios and compared with PEIs and Lipofectamine$^{TM}$. *P<0.05 vs PEI and Lipofectamine$^{TM}$.

3.9. Intracellular localization

Once successful transfection is achieved, it is important to track the path of cellular entry of the copolymer. For intracellular localization of dual labeled BL 2:1(15%)/DNA complex, HeLa cells were incubated with these complexes for specified time intervals, fixed, stained with DAPI and examined by confocal laser scanning microscopy. Both red and green fluorescence [red for BL 2:1(15%)-TMR and green for DNA-YOYO-1] were observed in the cytoplasm and nucleus of the cell within 1 and 2h of addition of the complexes, respectively (Fig. 8). These observations clearly demonstrate the efficient intracellular delivery of DNA using BL 2:1(15%), which is in agreement with a previous study reporting that PEI delivers nucleic acids to the nucleus.

3.10. In vivo gene expression

To validate the in vitro transfection by BL 2:1(15%)/DNA complex, it is pertinent to validate the same in vivo. Following intravenous injection of BL 2:1(15%)/DNA complex, naked DNA and bPEI/DNA complex in Balb/c mice, luciferase gene expression was measured in these groups post 7 days injections. It was observed that a significant higher luciferase expression (P<0.05) was exhibited by BL 2:1(15%)/DNA complex as compared to PEI/DNA complex and naked DNA with maximum expression in spleen followed by heart and brain. An insignificant gene expression was observed with naked DNA group, which could be due to degradation of the DNA by the nucleases present in the blood. However, in other organs like lungs, liver and kidney, appreciable luciferase expression was not observed (Fig. 9). Previous studies on the biodistribution of polymers also support these findings of observing maximum gene expression in spleen.
Figure 8: Confocal microscopic images of HeLa cells treated with TMR-BL 2:1 (15%)/YOYO-1-pDNA complex at different time points.

Figure 9: In vivo gene expression analysis in Balb/c mice 7 days post intravenous injection using pGL3 control vector as a reporter gene. *P<0.05 vs bPEI and DNA in the respective organs.
4. References


